Neuron
Review

Neural Stem Cell Biology in Vertebrates and Invertebrates: More Alike than Different?

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DOI 10.1016/j.neuron.2011.05.016

Many of the regulatory mechanisms controlling neural stem cell behavior are proving to be conserved between organisms as diverse as worms and man. Common principles are emerging with respect to the regulation of neural stem cell division and the specification of distinct stem and progenitor cell types. Great progress has been made in recent years in identifying the cellular mechanisms underpinning these processes, thanks in large part to the cross-fertilization of research on different model systems. We review here recent findings that highlight hitherto unappreciated similarities in the cell and molecular biology of neural stem cell self-renewal and differentiation between invertebrates and vertebrates. As well as underscoring the possible conservation of stem cell mechanisms across phyla, these similarities are proving to be practically useful in studying neural stem cell biology in health and disease.

Introduction

Stems cells are defined by their ability to divide in a self-renewing fashion to maintain a pool of stem cells while producing a continuous supply of differentiating daughters for the generation, and subsequent repair, of multiple tissues and to produce daughter cells for tissue homeostasis (reviewed in Weissman, 2000; Weissman et al., 2001). Discovering how stem cells control their multipotent state and how their progeny differentiate into distinct cellular fates is of fundamental importance, not only to understanding development, but also for understanding the pathogenesis of neurodevelopmental conditions, the initiation of brain tumors, and the therapeutic potential of stem cells. This is particularly important when considering the repair and regeneration of the nervous system after damage or disease.

Our understanding of nervous system development and neural stem cell biology has progressed rapidly in the past decade, thanks in large part to studies on invertebrate model systems. In particular, the *Drosophila* central nervous system (CNS) has served as a key model system in studying the asymmetric divisions of stem cells and, more recently, the link between unregulated stem cell division and tumorigenesis. The conservation of key aspects of the genetics of neural development among species has been appreciated for some time. Recent findings serve to emphasize the deep homologies between forebrain regions from species as diverse as humans and annelids: remarkably, the mushroom body of *Platynereis dumerilii* has been shown to share a "molecular fingerprint" with the developing mammalian cortex (Tomer et al., 2010). What has been particularly exciting recently has been the development of our understanding of the similarities between fundamental aspects of neural stem cell biology in *Drosophila* and in the mammalian cerebral cortex, the most highly evolved region of the mammalian CNS, in health and disease.

Neural Stem Cell Types in *Drosophila* and Mammals

Cellular diversity in the CNS is achieved by the regulated differentiation of multipotent neural stem cells. To date, three types of neural stem cells (or neuroblasts) have been described in the *Drosophila* brain and ventral nerve cord. Until recently, the general view was that *Drosophila* neuroblast types were very different from the stem cell types found in the polarized, pseudostratified neuroepithelia of the vertebrate CNS, including the cerebral cortex. However, striking parallels have emerged between the composition and organization of the optic lobe neuroepithelium and that of the mammalian cerebral cortex, as well as notable similarities in the division patterns and lineage outputs from neural stem cells in flies and mammals.

Type I neuroblasts account for the majority of stem cells in the *Drosophila* brain, with approximately 90 in each brain lobe, and until recently were considered to be the only stem cell present in the brain. At each cell division, type I neuroblasts generate a large neuroblast and a smaller daughter cell, called a ganglion mother cell or GMC (Figure 1). Type I neuroblasts express Deadpan (Dpn), a bHLH protein related to the vertebrate Hes family, and segregate the homeodomain transcription factor, Prospero (Pros; the ortholog of vertebrate Prox1), to their differentiating daughters. Mapping Prospero's targets throughout the genome has shown that Prospero directly binds and represses neuroblast genes and cell-cycle genes and is required to activate differentiation genes (Choksi et al., 2006). As a result, GMCs divide only once to produce two postmitotic neurons or glial cells.

By contrast, type II neuroblasts, of which there exist only eight per brain lobe, divide to give a neuroblast and a transit-amplifying cell called an intermediate neural progenitor (INP) (Bayraktar et al., 2010; Bello et al., 2008; Boone and Doe, 2008; Bowman et al., 2008; Weng et al., 2010). Type II neuroblasts express Deadpan, but not Prospero, and their daughters (INPs) lack Prospero protein. Furthermore, Asense (Jarman et al., 1993),

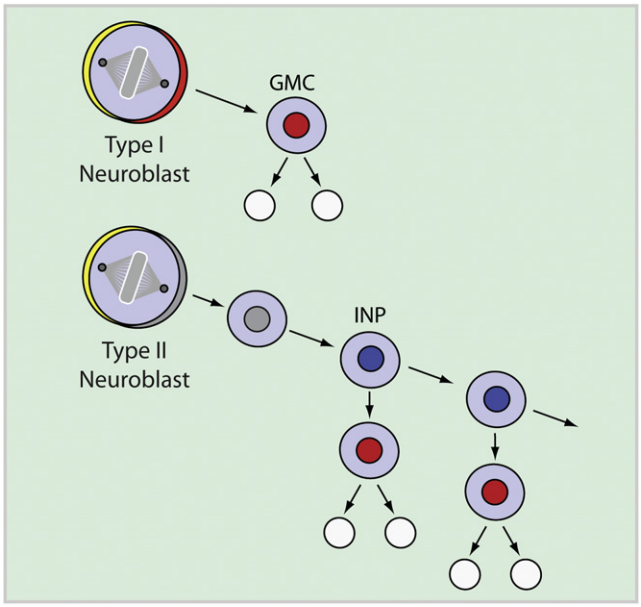

Figure 1. *Drosophila* Neural Stem Cells
Type I neuroblasts undergo multiple asymmetric divisions, whereby they self-renew while producing daughter cells (GMCs or ganglion mother cells) that divide only once to give two postmitotic neurons or glial cells. Type II neuroblasts divide to give a neuroblast and an intermediate progenitor (INP) that self-renews while producing GMCs. Type II neuroblasts generate much larger cell lineages than type I neuroblasts. The Par proteins are labeled in yellow; the cell-fate determinant, Prospero, is labeled in red; the Hes family transcription factor, Deadpan, is labeled in blue; neurons are labeled in white. Modified from Boone and Doe (2008).

a basic-helix-loop-helix (bHLH) protein and homolog of the vertebrate neural stem cell factor Ascl1 (Mash1), is expressed in most larval brain neuroblasts but is markedly absent from type II neuroblasts and immature INPs, which undergo multiple cell divisions (Bayraktar et al., 2010; Bowman et al., 2008; Weng et al., 2010). Misexpression of Ase appears to be sufficient to transform type II into type I neuroblasts (Bowman et al., 2008).

INPs divide from four to eight times, generating another INP and a GMC that divides only once (Figure 1). As a result of the self-renewing divisions of the INPs, type II neuroblasts generate much larger cell lineages than type I neuroblasts. Despite the differences in lineage output size, the division patterns of type I and type II neuroblasts are both similar to those seen in the mammalian cerebral cortex: apical stem cells in the cortex divide to generate another apical stem cell and either a neuron or a basal progenitor cell, with the latter typically dividing once to generate two postmitotic neurons (Figure 2) (Haubensak et al., 2004; Miyata et al., 2004; Noctor et al., 2004).

The third type of neuroblast is found in the optic lobe of the larval brain, where neural stem cells divide symmetrically within a pseudostratified neuroepithelium and are gradually converted to asymmetrically dividing neuroblasts in response to a wave of proneural gene expression (Egger et al., 2007, 2011; Hofbauer and Campos-Ortega, 1990; Yasugi et al., 2008). Again, there are striking parallels here with cortical apical progenitor cells, which form a polarized pseudostratified neuroepithelium and generate neurogenic basal progenitor cells that exit the pseudostratified neuroepithelium (Noctor et al., 2004).

During embryogenesis, neuroblasts can be identified by their unique combination of gene expression pattern and time and place of birth. As each neuroblast divides, it expresses a series of "temporal transcription factors" that dictates the identity of the neurons and glia in the lineage (Brody and Odenwald, 2000; Isshiki et al., 2001; Kambadur et al., 1998; Novotny et al., 2002; Pearson and Doe, 2003). At the end of embryogenesis most neuroblasts stop dividing and either undergo apoptosis or remain quiescent until larval stages. Postembryonic neuroblasts then resume division during larval and pupal stages to produce the majority of the neurons present in the adult CNS (Prokop and Technau, 1991). These neuroblasts provide an attractive model to study the transition from stem cell quiescence to reactivation.

Until recently, it was thought that no further cell division takes place in the *Drosophila* adult brain. However, two reports identified small numbers of dividing cells in the adult brain (Kato et al., 2009; von Trotha et al., 2009). The majority of these cells express the glial marker, Repo, and as yet there is no evidence for adult neurogenesis. An intriguing suggestion from observations of the adult hippocampus is that neural stem cells may eventually differentiate into postmitotic astrocytes. This would serve to explain the loss of stem cells and reduction in neurogenesis with age (Encinas et al., 2011). Might the Repo-expressing cells in the adult *Drosophila* brain be the end state of the neural stem cell lineage?

The *Drosophila* nervous system is an excellent model system in which to analyze the mechanisms controlling stem cell proliferation and differentiation at single-cell resolution. Given the recent insights into the similarities between *Drosophila* neuroblast types and mammalian cortical stem and progenitor cells, it will be interesting to explore whether that conservation extends to the cellular and molecular mechanisms regulating self-renewal, proliferation, and cell-fate decisions.

Multipotency and the Temporal Order of Neurogenesis

Key aspects of the biology of neural stem cells are their multipotency and the ability to generate complex lineages in a fixed temporal order. The multipotency of neural progenitor cells is inextricably linked with the fundamental problem of maintaining the balance between stem cell self-renewal and neurogenesis. Such a balance is essential for the generation of the correct proportions of different classes of neurons and subsequent circuit assembly: altering the balance toward excess neurogenesis will generate too few neurons by extinguishing lineages inappropriately early, whereas excessive self-renewal has the potential to lead to tumorigenesis.

A now classic transcription factor series expressed in neuroblasts in *Drosophila* has been identified as controlling the temporal order of neurogenesis in the embryonic central nervous system. Neuroblasts generate distinct neuronal and glial subtypes over time. This is achieved by the sequential expression of "temporal transcription factors": Hunchback (Hb), Kruppel (Kr), Pdm, Castor (Cas), and Grainyhead (Grh) (Brody and Odenwald, 2000; Isshiki et al., 2001; Kambadur et al., 1998; Novotny et al., 2002; Pearson and Doe, 2003). As each GMC is

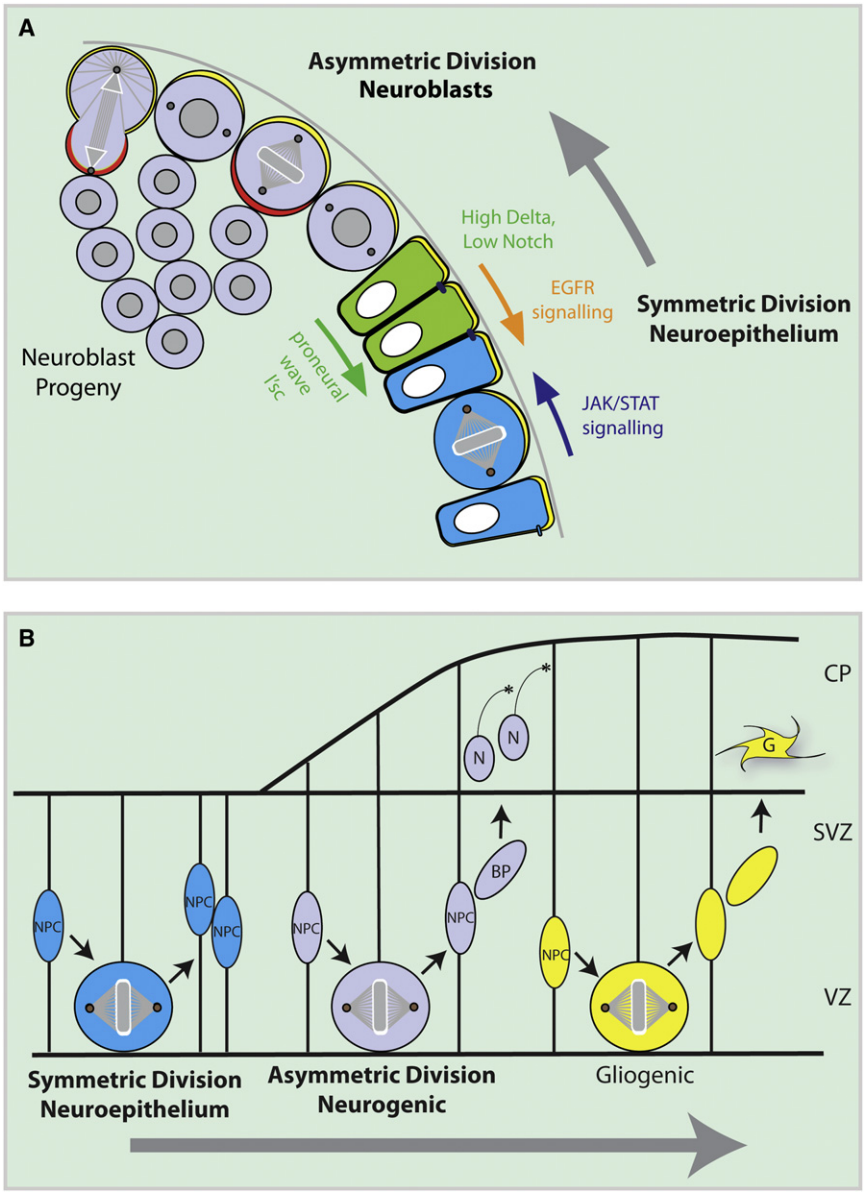

Figure 2. Cell Division in the *Drosophila* Optic Lobe and the Mammalian Cerebral Cortex

(A) During larval development the optic lobe of the brain consists of a pseudostratified neuroepithelium (light blue) in which stem cells divide symmetrically to expand the pool of proliferating precursor cells and of a region of asymmetrically dividing neuroblasts (gray), which produce the differentiated neurons that will make up the visual processing center of the brain. Neuroepithelial cells transform into neuroblasts in a highly ordered, sequential manner in response to a wave of proneural gene expression (green) that sweeps across the neuroepithelium. EGF signaling (orange) progresses the proneural wave, while JAK/STAT signaling (dark blue) inhibits it. In the transition zone (green), levels of Delta are high and Notch activity is low. The Par proteins are labeled in yellow; the cell-fate determinant, Prospero, is labeled red. Developmental progression is indicated by the gray arrow. Modified from Egger et al. (2007) and Egger et al. (2010).

(B) In the developing cortex, neural progenitor cells (NPC) divide symmetrically within a neuroepithelium (light blue). NPCs progress to asymmetric division (gray), generating a NPC and a basal progenitor (BP) or immature neuron. Neurons migrate out of the ventricular zone (VZ) to the cortical plate (CP) and basal progenitors move to the subventricular zone (SVZ). BPs divide further to generate neurons (gray). In the final phase of division, neural progenitor cells differentiate into glia (yellow). Developmental progression is indicated by the gray arrow. Modified from Kageyama et al. (2009).

born it continues to express the transcription factor present at its birth, and this expression pattern is thought to influence the neuronal and glial composition of the sublineage. Similarly, the temporal order of neurogenesis in the vertebrate retina and cerebral cortex is largely a cell-autonomous property of neural progenitor cells that can be recapitulated in vitro (Belliveau et al., 2000; Qian et al., 2000; Shen et al., 2006). The mammalian neocortex consists of six layers of neurons and glia (reviewed in Jacob et al., 2008; Okano and Temple, 2009). Each neural progenitor contributes progeny to all six layers, producing a number of different cell types in a distinct temporal order. The deepest layer of neurons forms first, and later-born neurons migrate progressively to the outer layers. Little is currently known of the control of the order of genesis in vertebrate neural lineages. However, the Ikaros transcription factor, one of the five vertebrate homologs of *Drosophila* hunchback, the first transcription factor in the sequence controlling the order of neurogenesis in flies, has been shown to regulate the genesis of early-born cell types in the mouse retina (Elliott et al., 2008). It is currently unknown whether this conservation of function extends to the cerebral cortex.

Drosophila neural stem cells transit through a period of quiescence separating distinct embryonic and postembryonic phases of proliferation (Hartenstein et al., 1987; Ito and Hotta, 1992; Prokop and Technau, 1991; Truman and Bate, 1988). During embryogenesis, neuroblasts primarily generate the neurons that make up the larval nervous system, while the progeny of the postembryonic neuroblasts populate the adult nervous system. Following the embryonic phase of proliferation, neuroblasts either enter into quiescence or undergo apoptosis. Quiescent neuroblasts reactivate and resume proliferation during larval and pupal stages, generating neurons that will contribute to the adult CNS (reviewed in Egger et al., 2008; Ito and Hotta, 1992; Prokop and Technau, 1991; Truman and Bate, 1988).

Quiescent neuroblasts, like quiescent neural stem cells of the mammalian SVZ and SGZ, exhibit a more complex morphology

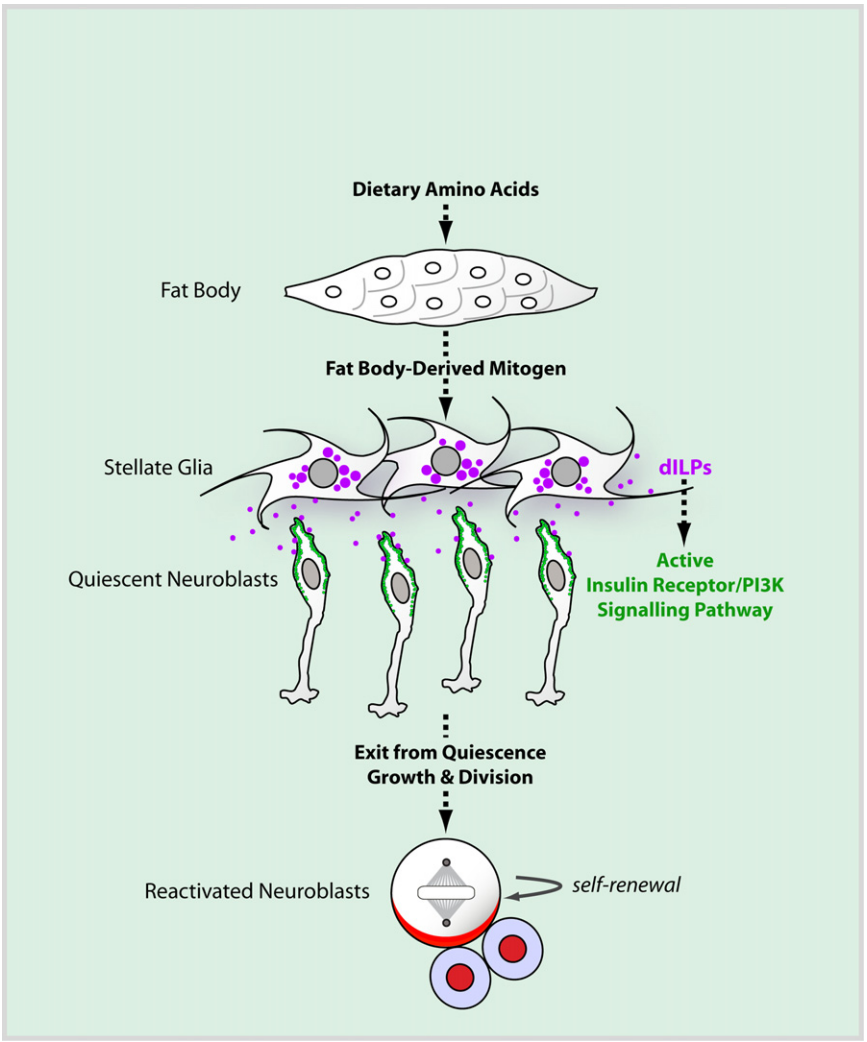

Figure 3. Reactivation of Quiescent Neural Stem Cells in *Drosophila*

A model for the nutritional control of neuroblast reactivation: dietary amino acids are sensed by the fat body, triggering secretion of a fat body-derived mitogen (FBDM). The FBDM induces surface glial cells to express and secrete insulin-like peptides (dILPs), which activate the dInR/PI3K/Akt pathway in neuroblasts. Activation of this pathway leads to cellular growth and cell-cycle re-entry. dILPs are in purple; active PI3K/Akt is in green; asymmetrically localized cell-fate determinants are in red. Modified from Chell and Brand (2010).

than proliferating cells (Figure 3) (Ma et al., 2009). They extend a primary cellular process toward the neuropil and also occasionally extend a process toward the ventral surface, or toward other neuroblasts (Truman and Bate, 1988). These processes are present until neuroblasts begin to divide (Chell and Brand, 2010; Tsuji et al., 2008), but their function has not yet been investigated.

Nutritional Control of Neural Stem Cell Exit from Quiescence

Systemic regulation ensures that stem cells meet the needs of an organism during growth, or in response to injury. A key point of regulation is the decision between quiescence and proliferation. In tissues such as blood, gut, and brain, stem cells spend much of their time in a quiescent, mitotically dormant state (for reviews see Ma et al., 2009; Moore and Lemischka, 2006; Woodward et al., 2005; Zammit, 2008). Neural stem cells in the mammalian subventricular and hippocampal subgranular zones transition between quiescence and proliferation, generating neurons throughout an animal's life (Ahn and Joyner, 2005; Doetsch et al., 1999; Ma et al., 2009; Morshead et al., 1994). Factors exhibiting mitogenic effects on neural stem cells have been identified, but it is not clear whether these factors act on stem cells or their proliferative progeny or at what point in the cell cycle these factors act (Zhao et al., 2008).

Britton and Edgar (Britton and Edgar, 1998) demonstrated that *Drosophila* neuroblasts exit quiescence in response to a nutrition-dependent signal from the fat body, a tissue that plays a key role in the regulation of metabolism and growth, but only recently have the molecules involved in reactivating neuroblasts been identified (Chell and Brand, 2010; Sousa-Nunes et al., 2011). To identify the signaling pathways involved in stem cell reactivation, Chell and Brand (2010) compared the transcriptomes of nerve cords containing either quiescent or reactivated neural stem cells, revealing that the expression of the insulin-like peptides dILP2 and dILP6 parallels stem cell reactivation. Furthermore, transcription of dILP6 increased 8-fold in response to a nutritional stimulus. The dILP6 promoter was found to drive expression in a set of stellate surface glial cells overlying the neuroblasts, suggesting that these glial cells might be the source of the signal that reactivates neuroblasts (Figure 3). Activity of the Insulin/IGF receptor pathway in neuroblasts was shown to be essential for neuroblasts to exit quiescence (Chell and Brand, 2010; Sousa-Nunes et al., 2011). In addition, the forced expression of insulin/IGF-like peptides in glia, or constitutive activation of PI3K/Akt signaling in neuroblasts, drove neuroblast proliferation in the absence of dietary protein, uncoupling neuroblast reactivation from systemic control.

IGF-1 and the PI3K/Akt pathway can also promote cell-cycle progression in vertebrate neural stem cells (Aberg et al., 2003; Mairet-Coello et al., 2009; Yan et al., 2006), suggesting that this same pathway may regulate vertebrate neural stem cell reactivation in a manner similar to that in *Drosophila*. In mammals, IGF-I can drive the proliferation of neural stem cells

in both the embryo and adult (reviewed in Anderson et al., 2002; Joseph D'Ercole and Ye, 2008). In response to injuries in the CNS, IGF-I expression is induced in stellate astrocytes (astroglia) (Yan et al., 2006; Ye et al., 2004) and is thought to be responsible for the increased neural stem cell proliferation observed in the subventricular zone and subgranular zone following cortical ischemia (Yan et al., 2006).

In the larval CNS, neuroblasts and their progeny are completely surrounded by glial cell processes. Glial cells also form part of the mammalian adult neural stem cell niche (reviewed in Nern and Momma, 2006) and astrocytes are thought to be a key component of the niches that dynamically regulate neural stem cell proliferation in the adult brain (Ma et al., 2005). Thus, much like mammalian astrocytes, *Drosophila* stellate glial cells perform a number of the functions that define a niche, and they control the proliferation of neural stem cells (Chell and Brand, 2010; Morrison and Spradling, 2008).

Symmetric and Asymmetric Stem Cell Division

In the nervous system, stem cells can divide symmetrically to generate daughter cells with similar fates, or asymmetrically, to self-renew while also producing differentiating daughter cells (Alvarez-Buylla et al., 2001; Temple, 2001). The proper balance between symmetric and asymmetric stem cell division is crucial both to maintain a population of stem cells and to prevent tumorous overgrowth. A body of recent work in vertebrates and invertebrates has highlighted the complexity of the mechanisms that regulate the balance between division types, ranging from well-known intercellular signaling pathways, such as Notch, to cell-cycle regulators and organelles such as the centrosome.

In the optic lobe of the developing *Drosophila* brain, symmetrically dividing neuroepithelial cells generate asymmetrically dividing neuroblasts, which produce the differentiated neurons that will make up the visual processing center of the brain (Figure 2) (Ceron et al., 2001; Egger et al., 2007, 2011; Hofbauer and Campos-Ortega, 1990). A comparison of the transcriptional profiles of neuroepithelial cells and neuroblasts revealed that genes in the Notch signaling pathway are preferentially expressed in neuroepithelial cells (Egger et al., 2010). Notch is required to maintain cells dividing symmetrically in the optic lobe neuroepithelium and prevent their switch to a neuroblast fate. Cells lacking Notch are extruded from the neuroepithelium and prematurely express the neuroblast-specific Hes family transcription factor, Deadpan (Dpn) (Egger et al., 2010; Ngo et al., 2010; Orihara-Ono et al., 2011; Reddy et al., 2010; Wang et al., 2011; Yasugi et al., 2010).

Inhibition of Notch also leads to premature differentiation in the mouse cerebral cortex where the maintenance of neural progenitors relies on oscillations in the expression of Notch target genes, such as Hes1, the ligand Delta-like1 (Dll1), and the proneural transcription factor Neurogenin2 (Ngn2) (Aguirre et al., 2010; Shimojo et al., 2008). Inhibition of Notch signaling leads to sustained expression of Dll1 and Ngn2 and to premature neurogenesis. Interestingly, high levels of Delta are found in the optic lobe at the transition zone separating the neuroepithelium from neuroblasts, where the levels of Notch are correspondingly reduced (Egger et al., 2010; Ngo et al., 2010; Orihara-Ono et al., 2011; Reddy et al., 2010; Wang et al., 2011; Yasugi et al., 2010). When expressed in the same cell, Delta and Notch can inhibit each other by *cis*-inhibition as opposed to *trans*-activation, which occurs between neighboring cells (Sprinzak et al., 2010). Delta may therefore inhibit Notch signaling at the transition zone, creating a sharp boundary between cells with mutually exclusive signaling states.

Neuroepithelial cells transform into neuroblasts in a highly ordered, sequential manner in response to expression of the proneural gene, *lethal of scute* (*l'sc*) (Yasugi et al., 2008). *l'sc* is expressed in the transition zone between the neuroepithelium and neuroblasts. A "proneural wave" of *l'sc* expression traverses the neuroepithelium, with cells ahead of the wave dividing symmetrically and those behind asymmetrically. Progress of the wave is regulated, at least in part, by the JAK/STAT and EGFR pathways (Yasugi et al., 2010, 2008). Yasugi et al. propose that the sequential activation of Notch and EGFR signaling drives the proneural wave forward, in a medial to lateral direction, while the JAK/STAT pathway negatively regulates its progression. Both Notch and EGFR signaling must be downregulated for the switch from neuroepithelial cell to neuroblast to occur (Yasugi et al., 2010).

The neuroepithelial to neuroblast transition in the optic lobe bears many similarities to the switch from self-renewing neuroepithelial cell to neurogenic radial glial cell in mammals (Farkas and Huttner, 2008; Gaiano et al., 2000; Heins et al., 2002; McConnell, 1995; Miyata et al., 2004; Noctor et al., 2004). In the optic lobe, Notch signaling maintains the neuroepithelial cell state and prevents neuroblast formation through direct cell-cell interactions. The EGFR and JAK/STAT pathways, activated by short-range and long-range signals, oppose each other and control the timing and progression of the neuroepithelial-to-neuroblast transition. In mammals the JAK/STAT pathway and the EGFR pathway regulate Notch activity in neural stem cells in vitro and in vivo. Notch activity promotes the neuroepithelial to radial glial cell transition (Gaiano et al., 2000), maintains radial glial cells in an undifferentiated state in the embryonic mouse brain through the interaction of Hes1 and Stat3 (Kamakura et al., 2004), and has recently been implicated in tumor initiation in mouse models of brain tumor development (Pierfelice et al., 2011). In the subventricular zone of the adult mouse brain, Notch maintains the neural stem cell state while EGFR signaling promotes more differentiated neural progenitors. A direct link between these pathways was recently discovered whereby EGFR signaling induces the ubiquitination and downregulation of Notch (Aguirre et al., 2010).

Cell-Cycle Regulation in Neural Stem Cells

The interplay between cell-cycle regulation and cell-fate determination in stem cells of the developing mammalian cerebral cortex is complex and bidirectional: signaling pathways and effectors that regulate cell-fate decisions can alter cell-cycle length, and regulators of the cell cycle can directly alter cell fate (Dyer and Cepko, 2000, 2001). The length of G1 phase was reported many years ago as increasing throughout development in cortical stem and progenitor cells (Takahashi et al., 1993, 1994). Subsequent work has shown that G1 lengthening acts to promote neurogenesis during development of the mammalian cerebral cortex (Lange et al., 2009) and is not

simply a passive consequence of the switch to neurogenesis. More recently, it has been found that the increase in G1 is due to an increase in the genesis of basal progenitor cells that have a relatively long G1 phase from apical progenitor cells that have a shorter G1 phase (Arai et al., 2011). In addition, an extended S phase is found in cortical stem cells that expand the stem cell pool as opposed to those destined to generate neurons. The latter observation has been suggested to reflect the greater need for careful quality control of DNA replication in expanding stem cells than in stem cells about to undergo a terminal division to generate two postmitotic neurons (Arai et al., 2011).

Optic lobe neuroepithelial cells also undergo a transient cell-cycle arrest prior to adopting the neuroblast fate (Hofbauer and Campos-Ortega, 1990; Orihara-Ono et al., 2011; Reddy et al., 2010). G1 arrest is induced through downregulation of the Fat-Hippo signaling pathway (Orihara-Ono et al., 2011; Reddy et al., 2010). Expression of a constitutively activated form of Yorkie (Yrk), a transcription factor controlled by Fat-Hippo signaling, prevents the cell-cycle arrest and blocks the transition from neuroepithelial cell to neuroblast. Similarly, in the chicken neural tube overexpression of Yes-associated protein (YAP, the vertebrate ortholog of Yrk) results in the expansion of the neural progenitor pool at the expense of differentiating cells (Cao et al., 2008). Recent results suggest that FatJ, the closest vertebrate homolog to *Drosophila* Fat, regulates Yap in the vertebrate neural tube (Van Hateren et al., 2011). In the *Drosophila* optic lobe as well as in the chicken neural tube YAP/Yrk positively regulates cell-cycle regulators to accelerate cell-cycle progression during early to mid-G1. Overall, it is clear that the complex interplay between cell-cycle regulation and cell-fate determination systems is a common feature of neural stem cells in vertebrates and invertebrates.

Asymmetric Segregation of Centrosomes in Neural Stem Cells

During asymmetric cell division in some cell types, the nonrandom segregation of mother versus daughter centrosomes has been observed to correlate with differences in cell fate (reviewed by Macara and Mili, 2008). The functional importance of centrosomes in neural stem cell self-renewal is evident from primary microcephaly (MCPH), an autosomal-recessive human condition in which the entire brain, and to a greater degree the cerebral cortex, are reduced in size (Thornton and Woods, 2009). Of the eight known MPCH loci, disease-causing mutations have been found in six genes, all of which encode proteins found in centrosomes (such as ASPM; Bond et al., 2002) or at the spindle pole (such as Wdr62; Bilgüvar et al., 2010; Nicholas et al., 2010; Yu et al., 2010). In radial glial cells in the developing mammalian cortex, the mother centrosome remains preferentially in the self-renewing cell, while newer centrosomes are segregated to differentiating daughter cells (Wang et al., 2009). Furthermore, removing one of the proteins required for centrosome maturation (ninein) disrupted orderly segregation and resulted in the loss of the self-renewing radial glial progenitor cells. These observations have led to the suggestion that the mother centrosome might confer stem cell properties on the cell in which it is retained.

Recently, however, research on neural stem cell division in the *Drosophila* larval brain has challenged this view (Conduit and Raff, 2010; Januschke et al., 2011). It transpires that in larval neuroblasts the mother centrosome is in fact inherited by the differentiating daughter cell, not the self-renewing cell. Conduit and Raff (2010) labeled centrosomes in vivo with GFP-PACT (a conserved centrosomal targeting motif in the coiled-coil proteins AKAP450 and pericentrin that is irreversibly incorporated into centrioles [Gillingham and Munro, 2000]) and, reasoning that centrosomal fluorescence should increase with age, were surprised to find that the brightest and presumably older centrosomes were inherited not by the neuroblast but by the GMC. Januschke et al. (2011) performed an elegant experiment that enabled them to identify unequivocally the old and new centrosomes (Figure 4). They labeled all centrioles of *Drosophila* neuroblasts with the photoconvertible fluorescent marker Eos fused to PACT and then photoconverted the mother centriole to emit red fluorescence so that it could be distinguished from the new centrioles, which remained green. The authors followed the differentially labeled centrosomes by time-lapse confocal microscopy and found that the old centrosome was segregated to the differentiating daughter cell while the self-renewing stem cell received the new centrosome. Interestingly, these results are similar to what is observed during cell division in budding yeast, where the daughter cell inherits the old centrosome (or spindle pole body) (Macara and Mili, 2008; Pereira et al., 2001). Januschke et al. (2011) propose that, rather than being associated with ''stemness,'' asymmetric centrosome segregation might be linked to life span. In each case, the ''most long-lived'' cell (yeast bud, male germline stem cell, and neuronal or glial daughters of the GMC) inherits the older centrosome.

Brain Tumor Initiation in *Drosophila* and Mammals

Misregulation of the mechanisms that control the balance between self-renewal and differentiation in neural stem cells has the potential to lead to brain tumor initiation. However, it has been challenging to identify the cell of origin for gliomas, the most common primary malignant brain tumor in humans. Recent studies, mainly in mouse models, strongly indicate these tumors arise from stem cells in the subventricular zone (Alcantara Llaguno et al., 2009; Jacques et al., 2010).

Many of the genes that regulate the asymmetric division of *Drosophila* neuroblasts, including Prospero, are known to act as tumor suppressors (Bello et al., 2006; Betschinger et al., 2006; Castellanos et al., 2008; Caussinus and Gonzalez, 2005; Choksi et al., 2006; Lee et al., 2006a; Lee et al., 2006b; Wang et al., 2007, 2006). Mutations in genes such as Prospero, Brat, and Numb lead to neuroblast overproliferation and result in brain tumors. Mutant brain cells can be transplanted into adult abdomens, where they continue to proliferate, begin to exhibit altered karyotypes, and can metastasize and eventually kill their host (Castellanos et al., 2008; Caussinus and Gonzalez, 2005). Conversely, genes that prompt neuroblast self-renewal, for example aPKC, are likely to act as oncogenes (Lee et al., 2006c). Identifying the transcriptional networks that regulate neural stem cell division is helping to elucidate the normal sequence of events that take place in the transition from stem

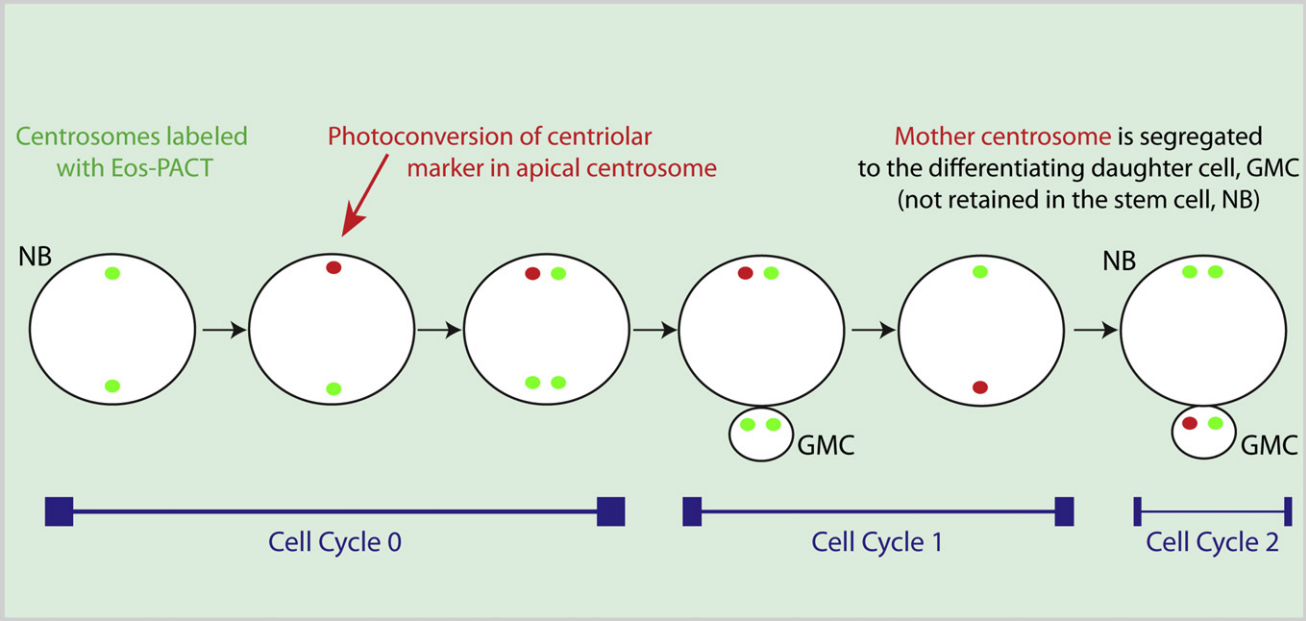

Figure 4. Asymmetric Centrosome Segregation
Differential labeling of the old (red) and new (green) centrioles in a dividing *Drosophila* larval neuroblast demonstrated unequivocally that the old centrosome is not retained by the neural stem cell but is instead inherited by the daughter cell. Modified from Januschke et al. (2011).

cell to differentiation (Choksi et al., 2006; Southall and Brand, 2009) and aid in identifying the changes that lead to tumor initiation.

In *Drosophila*, overproliferation of the optic lobe neuroepithelium also gives rise to tumors. Janic et al. (2010) studied the effect of mutations in the gene *l(3)mbt* (malignant brain tumor) (Gateff et al., 1993) on the developing brain. *l(3)mbt* is most closely related to the polycomb group proteins and, with the two *Drosophila* Retinoblastoma family proteins, forms part of the dREAM-MMB complex (Bonasio et al., 2010; Lewis et al., 2004). Consistent with this the human ortholog, L3MBTL1, is a transcriptional repressor that is found associated with core histones, the retinoblastoma protein, and heterochromatin protein 1 gamma (HP1gamma) (Boccuni et al., 2003; Trojer et al., 2007). While a role in tumorigenesis for the human orthologs of MBT has not been found to date (Bonasio et al., 2010), increased polycomb activity, and particularly increased activity of the PRC2 complex histonemethyltransferase Ezh2, is a key element in glioblastoma progression (Lee et al., 2008).

As is evident from its name, mutations in *l(3)mbt* cause tumorous overgrowth in the larval brain, generating brains that are seven times larger than normal. To discover which genes might account for this malignant growth, Janic et al. (2010) assessed the transcriptional profile of the tumor cells. Remarkably, when they surveyed the transcriptome of the *l(3)mbt* tumors, they found that a large number of germline genes were ectopically expressed. Their results implied a soma-to-germline transformation in the brain.

Interestingly, not all *Drosophila* brain tumors exhibited the same transcriptional profile as the *l(3)mbt* tumors. When Janic et al. (2010) profiled tumors arising from mutations in the genes *brat*, *lethal giant larvae*, *miranda*, *prospero*, or *pins*, they found the distinctive germline signature only in the *l(3)mbt* tumors. Of the 102 genes specifically upregulated in response to the *l(3)mbt* mutation, 26 are normally required in the germline. Even more remarkably, the authors found that the *l(3)mbt* tumors can be suppressed by removing individually any one of four germline genes: *piwi*, *aub* (both involved in the biogenesis of piRNAs) *vasa* (required for the assembly of pole plasm and for germline development), or *nanos* (involved in the establishment of pole plasm). Of these, *piwi* and *nanos* are homologous to so-called "cancer testis" or "cancer-germline" genes, which are expressed ectopically in several human malignancies (Simpson et al., 2005).

Making Neurons Directly and Indirectly from Nonneural Cell Types: Cellular Reprogramming

The isolation of neural stem cells (Gage, 2000), the advent of induced pluripotent stem cells (iPS) (Takahashi and Yamanaka, 2006; Yamanaka, 2009), and the subsequent generation of neurodegenerative disease-specific iPS (Dimos et al., 2008; Ebert et al., 2009; Park et al., 2008; Soldner et al., 2009) has raised the prospect of treatment for disorders such as Alzheimer's disease, amyotrophic lateral sclerosis (ALS), spinal muscular atrophy (SMA), Parkinson's disease, Huntington disease, and spinal cord injury. A deep understanding of the cell and molecular biology of neural stem cells continues to be essential to the rational exploitation of these systems for generating specific cell types and ultimately the construction of brain circuits for tissue engineering.

An exciting advance in this area was the discovery that the combined expression of only three transcription factors, Ascl1,

Brn2 (also called Pou3f2) and Myt1l, is sufficient to convert fibroblasts into postmitotic neurons without the need for cell-cycle progression (Vierbuchen et al., 2010). Not only do the neurons induced by these neural lineage-specific factors express neural proteins, but they are also able to form synapses and to generate action potentials and are thus definitively functional neurons (referred to as induced neurons, or iN cells). This landmark work has established the principle that nonneural cells can be directly transdifferentiated or reprogrammed to functional neurons. Currently, one of the hurdles for reprogramming has been the efficiency with which the desired cell type can be produced, with efficiencies of up to 19.5% observed. A further technical challenge to be overcome is the ability to generate defined classes of neurons in an efficient, controlled manner.

In a striking in vivo parallel to the iN work, Tursun et al. (2011) found that mutating a single gene in *C. elegans*, encoding the histone chaperone LIN-53 (a homolog of the human retinoblastoma binding protein, RbAp46/48 [Lu and Horvitz, 1998]), enabled germ cells to be converted into neurons. In the *lin-53* mutant background, expression of a single transcription factor could transform germ cells into a specific, identifiable neuronal subtype.

One of the transcription factors the authors misexpressed, CHE-1, specifies the identity of a pair of gustatory neurons known as ASE neurons. CHE-1 has been shown to bind to many of the genes required to generate the terminal phenotype of the ASE neurons (Etchberger et al., 2007; Uchida et al., 2003). Although misexpression of CHE-1 was sufficient to activate transcription of a synthetic reporter gene, when CHE-1 was misexpressed postembryonically, it was only able to activate some ASE markers in a small number of head sensory neurons. The authors screened an RNAi library to identify genes that, when knocked down, would allow more extensive cellular reprogramming. The authors found that when lin-53 was knocked down, expression of CHE-1 was sufficient to convert nonneuronal cells into cells expressing ASE cell-fate markers. Numerous ASE-like neurons were discovered in the gonad, where germ cells had been reprogrammed. The reprogrammed cells expressed a battery of genes normally transcribed in ASE neurons, but not those associated with other neuronal subtypes (dopaminergic, serotonergic, cholinergic, or GABAergic markers). The germline cells could be converted to other subtypes of neurons after expression of the appropriate neuronal-specific transcription factor, such as unc-30 to express GABAergic markers, or unc-3 to generate cholinergic A/B-type ventral cord motor neurons. Interestingly, a muscle-specific transcription factor was unable to convert germ cells to a muscle cell fate, suggesting, perhaps, that other chromatin factors might be involved, to recruit different subsets of histone modifiers or remodellers.

Conclusions

Studies of neural stem cell biology in model organisms, both vertebrate and invertebrate, have revealed underappreciated similarities in the regulation of self-renewal, multipotency, and cell-fate determination. The ability to carry out precise genetic manipulation in *Drosophila* neural stem cells, compared with vertebrates, has facilitated insightful exploration of novel mechanisms regulating neural stem cell proliferation under normal conditions and in disease. The latter has led to the development of very useful models of brain tumor initiation in flies that are now being explored with the unparalleled genetic toolkit available for *Drosophila.* As in vitro mouse and human systems based on iPS and transdifferentiation become more widely used, it will be fascinating to use the complementary strengths of vertebrate and invertebrate systems to answer some of the pressing questions in the biology of neural stem cells and explore their therapeutic potential.

ACKNOWLEDGMENTS

A.H.B. is supported by a Wellcome Trust Programme Grant; F.J.L. is supported by the MRC, the Wellcome Trust, and Alzheimer's Research UK; A.H.B. and F.J.L. are supported by a core grant from the Wellcome Trust and Cancer Research UK. Many thanks to Elizabeth Caygill, James Chell, and Boris Egger for figures and for comments on the manuscript, and to the anonymous reviewers for helpful comments.

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